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Processing plant and machinery sanitation and hygiene practices associate with *Listeria monocytogenes* occurrence in ready-to-eat fish products

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ABSTRACT

Listeria monocytogenes causes the foodborne illness listeriosis, which exhibits high fatality among people in risk groups. The incidence of listeriosis has increased in Europe, which raises concerns about *L. monocytogenes* occurrence in foodstuffs. Ready-to-eat seafood products are considered particularly risky vehicles. Poor hygiene at processing facilities predisposes them to *L. monocytogenes* contamination, which can be controlled by stringent self-checking system measures. We examined the association of fish-processing plant operational and hygiene practices with the occurrence of *L. monocytogenes* in vacuum-packaged gravad (cold-salted) and cold-smoked salmon and rainbow trout products. Product sampling of 21 fish-processing plants was carried out, and operational procedures relating to *L. monocytogenes* control were surveyed using an in-depth risk assessment questionnaire. *L. monocytogenes* occurred only in sliced and mainly in gravad products of seven fish-processing plants. Shortages in preventive measures were discovered predominantly among the *L. monocytogenes* positive fish-processing plants. Using generalized linear modeling, we identified the following features associated with *L. monocytogenes* product contamination: the number of processing machines, deficiencies in the processing environment and machinery sanitation, and staff movement from areas of low toward high hygiene. Furthermore, performing frequent periodic thorough sanitation alongside everyday sanitation practices associated with a decreased risk of product contamination.

1. Introduction

The foodborne illness listeriosis caused by the bacterium *Listeria monocytogenes* has become increasingly prevalent in Finland and other parts of Europe in recent years (European Food Safety Authority and European Centre for Disease Prevention and Control, 2017; National Institute for Health and Welfare, 2018). Listeriosis is fatal in 20–30% of cases, and risk groups include neonates, pregnant women, the elderly, and immunocompromised individuals (Vazquez-Boland et al., 2001). The rising incidence of this severe disease can be partly due to the increase of susceptible populations but also raises concerns about the occurrence of *L. monocytogenes* contaminated foodstuffs among products available for consumption (Goulet et al., 2008; Ricci et al., 2018). In the European Union (EU), the microbiological criteria concerning *L. monocytogenes* in foodstuffs are stringent for products which can support the growth of *L. monocytogenes* or are intended for the populations at risk (EC No 2073/2005). *L. monocytogenes* grows both in aerobic and anaerobic conditions, and in a wide temperature and pH range (−1.5–45 °C; pH 4.3–9.6) and withstands up to 10% salinity (Gray and Killinger, 1966; Junttila et al., 1988). These factors impede the control of the bacterium in the food chain through the traditional means of salting, refrigerating, and modified atmospheric packaging. Vacuum-packaged ready-to-eat (RTE) fish products such as gravad (cold-salted) and cold-smoked products that do not undergo listericidal thermal processing can contain *L. monocytogenes* (Åberg et al., 2008; Kramarenko et al., 2016; Niskanen et al., 2010) and are considered particularly risky foods for contracting the disease (Ericsson et al., 1997; Gillesberg Lassen et al., 2016; Miettinen et al., 1999; Nakari et al., 2014).

L. monocytogenes occurs in soil and water and hence on fish farms (Miettinen and Wirtanen, 2006), from where raw fish can carry the bacterium into the fish-processing chain (Eklund et al., 1995; Farber, 1991; Markkula et al., 2005). *L. monocytogenes* typically contaminates products via food processing facilities by persistent contamination in processing environments when favorable conditions occur (Autio et al., 2004; Blatter et al., 2010; Di Ciccio et al., 2012; Lundén et al., 2003).

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Representative places for *L. monocytogenes* contamination in fish-processing plants (FPPs) include processing machinery, surfaces, transporters, utensils, brine, floors, drains, and personnel work clothing (Autio et al., 1999; Gudmundsdottir et al., 2005; Thimothe et al., 2004; Vogel et al., 2001). Thereby, unhygienic processing practices and poor maintenance and sanitation of facilities contribute to fish product contamination with *L. monocytogenes* (Miettinen et al., 2001; Rørvik et al., 1997). The occurrence of *L. monocytogenes* in FPPs can be reduced by rigorous interventions targeting hygiene and working practices (Autio et al., 1999; Lappi et al., 2004). The current ways of managing food safety risks at FPPs include good manufacturing practices, sanitation standard operating procedures, and hazard analysis and control principles such as 'hazard analysis and critical control points', i.e., HACCP (European Salmon Smokers Association, 2018). FPP self-checking systems (management systems), which are a compilation of plans, self-surveillance, and execution of prerequisite programs and hygienic protocols, are therefore crucial for *L. monocytogenes* prevention and are required by law (EC No 2073/2005; EC No 852/2004; EC No 853/2004; EC No 854/2004).

Despite the apparent risk of *L. monocytogenes* contamination in facilities and on food contact surfaces of FPPs (Di Ciccio et al., 2012; Lappi et al., 2004; Nakamura et al., 2006; Summa et al., 2016), the current inclusion and implementation of *L. monocytogenes* preventive measures in various FPPs has not been extensively studied. Although drivers of product contamination have been investigated (Lappi et al., 2004; Rørvik et al., 1997; Rotariu et al., 2014), more in-depth knowledge of concrete present-day production practices and execution of preventive measures is required in order to enhance *L. monocytogenes* prevention in FPPs. Using an in-depth inspection questionnaire, we investigated the association of FPP production and hygiene practices with *L. monocytogenes* product contamination in a third of the Finnish FPPs producing vacuum-packaged RTE products in 2014–2015. We aimed to determine the current implementation of *L. monocytogenes* preventive measures in the FPPs and identify ways in which the measures could be improved. Our investigation discovered practices that are associated with an increased or decreased risk of *L. monocytogenes* product contamination in fish-processing.

2. Materials and methods

2.1. L. monocytogenes product sampling

The occurrence of *L. monocytogenes* in vacuum-packaged RTE gravad (cold-salted) and cold-smoked fish products of 21 Finnish FPPs was studied during a 14-month period between September 2014 and October 2015. Of the studied FPPs, all 21 produced cold-smoked products and 18 also produced gravad fish using rainbow trout and salmon. A sampling of retail-packaged fish products from each FPP was carried out at approximately 2-month intervals. The vacuum-packaged products were obtained from FPPs and transported on ice via express post. The samples were stored at 3 °C until their end of shelf life, i.e., the storage time defined by the production and use-by dates, as determined by the manufacturer. Detection and enumeration of *L. monocytogenes* were performed at the Finnish Food Safety Authority Evira (renamed the Finnish Food Authority as of January 1st, 2019) using International Organization for Standardization methods ISO/DIS 11290-1 and 2 (ISO, 2014a; ISO, 2014b). By assembling cuts from several parts of the product, a 100–150 g sample was homogenized, and 25 g (detection) or 10 g (enumeration) of the homogenate were used for the analyses. The presumptive L. *monocytogenes* were confirmed using the API Listeria kit (bioMérieux, Marcy l'Etoile, France). Isolates was stored at −70 °C in brain heart infusion with 15% glycerol. Pulsed-field gel electrophoresis (PFGE) typing was performed using the enzymes *Asc*I and *Apa*I (Roussel et al., 2014). The status of *L. monocytogenes* as "positive" or "negative" was assigned to each FPP based on whether their product samples were contaminated with *L. monocytogenes* during the investigated period.

Table 1
Topics included in the risk assessment questionnaire.

Topic	Number of questions
Fish-processing plant and inspector background	44
Fish-processing plant self-checking system	8
Compartmentalization of production steps and division of the plant into hygiene levels	43
Routes used by staff, materials and products	22
Hygiene of processes and personnel	43
Timing and extent of sanitation procedures	21
Dismantling and sanitation of processing machines	110
Contact surface condition and cleanliness	66
Processing parameters for raw materials, salting, and smoking	62
Listeria monocytogenes sampling of processing machinery	18
Shelf life of products	11
Questions in total	448

2.2. Risk assessment questionnaire

In order to assess the prerequisites for managing *L. monocytogenes*, an in-depth inspection protocol in the format of a risk assessment questionnaire was devised for the 15 official inspectors of the 21 participating FPPs. A total of 448 questions included queries on background information and topics that covered FPP practices and production procedures relating to *L. monocytogenes* control (Table 1). We asked processing-step-specific questions concerning the processing environment and machinery, implementation of manufacturing processes including hygiene and sanitation practices, and opinions of the inspector on FPP compliance. The answers were quantitative (numeric), qualitative (yes–no), or Likert scale for opinion (1–4: completely agree – somewhat agree – somewhat disagree – completely disagree), frequency (1–6: always – often – quite often – quite seldom – seldom – never), and extent (1–6: not at all – little – quite little – quite much – much – very much).

The questionnaire comprised two parts: an online form for the background information and opinions of the inspector, and a processing step-by-step printable text file to be filled in during an official inspection visit to the FPP, where the inspectors based their evaluations on the existing food safety legislation and competent authority guidelines applicable to Finnish FPPs (EC No 178/2002; EC No 852/2004; EC No 853/2004; EC No 854/2004; EC No 882/2004; Ministry of Agriculture and Forestry statute 795/2014; EC No, 2073/2005; EC No 852/2004; EC No 853/2004; EC No 854/2004; EC No 882/2004; Finnish Food Safety Authority Evira, 2009; Finnish Food Safety Authority Evira, 2010; Finnish Food Safety Authority Evira, 2014; Finnish Food Safety Authority Evira, 2015a; Finnish Food Safety Authority Evira, 2015b; Food Act, 2006; Ministry of Agriculture and Forestry statute 795/2014). The inspectors performed the visits and returned the questionnaires in June–October 2015.

For each FPP, the questionnaire answers were either directly used as variables (e.g., presence of a processing step, machine, or procedure) or were summarized as average variables from Likert scale questions on opinions (for which Cronbach's alpha was over 0.7; Supp. Table S1) and the same questions concerning a topic (e.g., processing machine sanitation) repeated throughout the different processing steps (Supp. Document S2). The answers available from each FPP depended on their processing steps – if for instance slicing was not performed, answers for this processing step were not obtained. The content of the summed average variables for each study participant thus consisted of the processing steps from which answers were obtained for that particular FPP.

2.3. Statistical analyses

In order to identify FPP practices and processing procedures

associated with *L. monocytogenes* occurrence in gravad and cold-smoked fish products, differences in the questionnaire variables between *L. monocytogenes* positive and negative FPPs were investigated in R (version 3.4.0) and SPSS (version 24). Our statistical protocol included consideration of multiplicity and sample size as follows. An exploratory approach was utilized for creating hypotheses from the multitude of collected variables (Armstrong, 2014; Streiner and Norman, 2011). Associations of individual variables with the *L. monocytogenes* status of the FPPs were preliminarily examined using either Fisher's exact test or the Mann–Whitney *U* test, as applicable, to decide which variables should be used as covariates and factors in the multivariate testing. For this initial creation of hypotheses, it was more important not to omit possible true associations (type II error) than to avoid including possible false ones (type I error), and thereby a correction for multiple significance testing was not required (Armstrong, 2014; Schulz and Grimes, 2005; Streiner and Norman, 2011). Subsequently, the process, procedure, processing machine, and hygiene parameters having $p \leq 0.1$ were used in the multivariate generalized linear modeling, i.e., logistic regression, with R package *logistf* to test the hypotheses generated from the individual variables. Quasi-complete separation occurred due to highly predictive combinations of variables in a small sample size (Heinze and Schemper, 2002), and thereby a correction of regression coefficients was performed using Firth's penalized method (Firth, 1993; Heinze and Ploner, 2004). Statistical models were fitted for two different subsamples: (a) using variables with data from all 21 FPPs and (b) using variables concerning processing machinery from 15 FPPs, all of which utilized a slicing machine. The latter was performed in order to investigate beyond the occurring quasi-complete separation (Albert and Anderson, 1984) and to identify which factors within the FPPs utilizing slicing and skinning machines contributed to *L. monocytogenes* product contamination.

Following the data exploration and generalized linear modeling analysis protocols of Zuur et al. (2009, 2010), collinearity was handled by examining Pearson and Spearman correlations, ensuring to only include variables with variance inflation factor < 3 in the model fitting. The variables initially included in the fitting of the two separate models (a and b) were: (a) “number of processing machines,” “frequency of periodic thorough sanitation of the processing environment,” and “assigned person in charge of each self-checking program (yes–no)”; and (b) “written sanitation plan for vacuum machine (yes–no),” “in-between-process cleaning of slicing machine (yes–no),” “staff movement from areas of low toward high hygiene during processing day (yes–no),” and “periodic thorough sanitation of the vacuum machine (yes–no).” Penalized likelihood ratio tests were used for backward selection of variables, which were removed as long as the residual deviance remained insignificant at the > 0.05 level (Heinze and Ploner, 2004). The explained deviance (pseudo-R^2 (Dobson, 2002), was determined for the final models, the first of which included two covariates and the second two factors.

3. Results

3.1. L. monocytogenes occurrence in fish products

In total, 425 vacuum packages of gravad and cold-smoked fish products from 6 to 18 production lots per FPP were collected (Table 2). As a whole, 4.2% of the sampled packages originating from seven different FPPs were contaminated with *L. monocytogenes*. The contamination level was below 10 cfu/g, except for one sample (20 cfu/g). The PFGE typing yielded a total of 10 different pulsotypes. The same pulsotypes did not occur in different FPPs, but in three FPPs where contamination recurred, the same pulsotype was found on multiple sampling occasions. Contamination only occurred in the sliced products (6.2%), among which *L. monocytogenes* was significantly more common in the gravad than cold-smoked products (11% vs. 1.9%, respectively, Fisher's exact test, $p = 0.001$, Table 3). Salt (NaCl) content was reported in the product labeling of 392 samples and ranged from 1.0% to 4.0% in the cold-smoked and from 1.5% to 4.0% in the gravad samples. The L. *monocytogenes* positive samples had a higher mean reported salt content (3.3%, 3.4%, 3.4%) than the negative samples (2.6%, 2.7%, 2.8%) within all, gravad, and gravad-sliced products, respectively (Mann–Whitney *U* test, $p \leq 0.005$).

3.2. Shelf life

The shelf life of the investigated fish products varied by FPP from 4 to 15 days and 8–21 days for the gravad and cold-smoked products, respectively. The most commonly given shelf life (48% of samples) was 14 days, which was the median shelf life for both *L. monocytogenes* positive and negative products. No statistical association was observed between the shelf life and *L. monocytogenes* product contamination. In 6/21 FPPs, the shelf life for sliced products was more than 14 days: three FPPs exceeded this by 1 day for gravad, but in five FPPs, the shelf life for cold-smoked products was up to 1 week longer. During the 14-month sampling, the shelf life given by 16/21 FPPs varied by 1–10 days (median 3) between the samples of the same type of product at different sampling occasions from the same FPP.

3.3. Processes and working hygiene in gravad and cold-smoked fish production

L. monocytogenes only occurred in the products of FPPs using skinning and slicing machines, which were the most common machinery in the studied FPPs in addition to the vacuum machine (Table 4). The skin of the fish was estimated to touch the flesh side of the fish, either directly or through contact surfaces, on average “not at all” or “little” within the processing machinery at the *L. monocytogenes* negative and positive FPPs, respectively (Table 5). On average, the *L. monocytogenes* positive FPPs had four processing machines, whereas the *L. monocytogenes* negative FPPs had two (Mann–Whitney *U* test, $p = 0.04$). The number of processing machines correlated with the FPP size (i.e. output in tons, Spearman's rho 0.8, $p < 0.001$) and remained a significant explanatory variable for *L. monocytogenes* contamination of products in the generalized linear modeling (Table 6).

The executed processing steps varied notably between the investigated 21 FPPs (Fig. 1 and Table 4). Dry salting was the most common way of salting: used by 17/18 and 14/21 FPPs producing gravad and cold-smoked fish, respectively. Submersion brining was used only for cold-smoked products in four FPPs, and injection brining was used in four FPPs, of which two used it for gravad products and all for the cold-smoked products. One FPP used liquid smoke and the rest traditional smoking by wood burning. No statistical associations emerged between the individual salting or smoking parameters and the *L. monocytogenes* status of the FPPs. A somewhat larger proportion of the FPPs, where fish skin was absent during the salting of gravad fish was *L. monocytogenes* positive compared with the FPPs, where skin had not been removed before salting, but this difference was not significant in the statistical analyses of the individual parameters (57 vs. 27%, respectively, Fisher's exact test, $p = 0.3$; Table 4).

On average, the inspectors were of the opinion that hands and utensils were washed and gloves changed “often” or “quite often” when required, and only minor differences appeared between the estimations for the *L. monocytogenes* positive and negative FPPs (Table 5). Assigning a person in charge of each FPP self-checking program appeared somewhat more common among the *L. monocytogenes* negative than positive FPPs (Table 4), but was not a significant factor in the multivariate analysis (chi-squared = 3.8, $p > 0.05$). Staff movement between processes from areas of low toward high hygiene during the production day occurred at 14 FPPs and was reported not to occur in 7 FPPs, of which 43% and 14%, respectively, were *L. monocytogenes* positive (Fisher's exact test, $p = 0.3$; Table 4). This difference in the proportion of the *L. monocytogenes* positive FPPs increased when including only the FPPs

Table 2
Summary of product sampling and occurrence of *Listeria monocytogenes* (*Lm*) in vacuum-packaged ready-to-eat gravad and cold-smoked fish products from 21 fish-processing plants (FPPs) in Finland.

FPP	Total production[a]	Number of sampling occasions per FPP[b]	Number of production lots tested	Number of tested packages (% of which sliced and gravad[c])	Number of *Lm* positive sampling occasions per FPP	Number of *Lm* positive production lots (%)	Number of *Lm* positive packages (%)
A	Large	7	18	21 (43)	1	1 (6)	2 (9.5)
B	Large	7	7	21 (43)	0	0 (0)	0 (0)
C	Large	7	7	21 (57)	2	2 (29)	2 (9.5)
D	Large	7	7	21 (0)	0	0 (0)	0 (0)
E	Large	6	6	18 (100)	3	3 (50)	3 (17)
F	Large	7	8	21 (57)	1	1 (13)	1 (4.8)
G	Medium	7	7	21 (0)	0	0 (0)	0 (0)
H	Medium	7	7	21 (57)	0	0 (0)	0 (0)
I	Medium	7	7	21 (43)	1	1 (14)	3 (14)
J[d]	Medium	7	7	21 (43)	2	2 (29)	5 (24)
K	Medium	7	7	21 (43)	0	0 (0)	0 (0)
L	Medium	7	16	21 (57)	0	0 (0)	0 (0)
M	Medium	7	15	21 (43)	0	0 (0)	0 (0)
N	Medium	7	6	21 (29)	1	1 (17)	2 (9.5)
O	Small	7	7	21 (0)	0	0 (0)	0 (0)
P	Small	5	7	21 (48)	0	0 (0)	0 (0)
Q	Small	7	13	21 (0)	0	0 (0)	0 (0)
R	Small	7	7	21 (0)	0	0 (0)	0 (0)
S	Small	7	7	21 (0)	0	0 (0)	0 (0)
T	Small	7	12	17 (0)	0	0 (0)	0 (0)
U	Small	5	7	12 (0)	0	0 (0)	0 (0)
Total	NA	5–7	185	425 (32)	1–3	11 (6)	18 (4.2)

[a] Large = > 1 000 000 kg/year; medium = 100 000–1 000 000 kg/year; small < 100 000 kg/year.
[b] Samples were collected at approximately 2-month intervals from each FPP during a 14-month period.
[c] The majority of the *Lm* positive products were sliced and gravad. The other tested product types included "non-sliced, gravad," "sliced, cold-smoked," and "non-sliced, cold-smoked."
[d] The only FPP where cold-smoked products (n = 3) were found positive.

Table 3
Percentage of *Listeria monocytogenes* positive vacuum-packaged cold-smoked and gravad fish products by sample type. Total amount of samples in each category is indicated in parentheses.

Sample type	Cold-smoked	Gravad
All samples	1.2^{a} (256)	8.9^{b} (169)
Sliced samples	$1.9^{a,A}$ (155)	$11^{b,A}$ (136)
Non-sliced samples	$0^{a,A}$ (101)	$0^{a,B}$ (33)

The proportions that do not differ significantly at the 0.05 level (Fisher's exact test) are marked by the same superscript lowercase and uppercase letter within rows and columns, respectively.

using a slicing machine (75% vs. 14%, respectively, Fisher's exact test, $p = 0.04$; Table 4), and the movement from areas of low toward high hygiene was found to significantly increase the risk of product contamination with *L. monocytogenes* (Table 6).

3.4. Sanitation of processing environment and machinery

All FPPs reported to clean and sanitize their production premises after each production day. Cleaning was executed by the staff in 16/21 and by an outside cleaning service in 7/21 of the FPPs, including four FPPs where cleaning was performed by both. Of the FPPs that performed cleaning procedures while processing was taking place, 50% were *L. monocytogenes* positive, as opposed to 27% of those that did not (Table 4). Mechanical cleaning was used on average "quite often" for processing machines, which were also estimated to be somewhat cleaner than the other processing surfaces (Table 5). Dismantling of the processing machinery during sanitation was performed on average "quite seldom" and "quite often" in the *L. monocytogenes* positive and negative FPPs, respectively (Table 5). In addition, *L. monocytogenes* self-checking sampling was extended to fewer machines in the *L. monocytogenes* positive than negative FPPs, including on average 21% vs. 35% of the machines, respectively (Table 5). No significant statistical associations with the *L. monocytogenes* status were observed in the analyses of the aforementioned individual variables.

In-between-process cleaning refers to cleaning the machinery during a processing day: e.g., during a break or before a new production lot. It was performed in 16/21 FPPs, where the methods of in-between-process cleaning included rinsing with water, disinfection, mechanical cleaning, or performing the regular sanitation process. Of the 7/16 FPPs that mentioned rinsing with water, 57% were *L. monocytogenes* positive, whereas 22% of those not mentioning it (9/16 FPPs) had *L. monocytogenes* contamination in their products (Fisher's exact test, $p = 0.3$). Conversely, none of the 5/16 FPPs that mentioned performing disinfection as part of their in-between-process cleaning were *L. monocytogenes* positive, while 55% of the 11/16 FPPs not mentioning it had *L. monocytogenes* product contamination (Fisher's exact test, $p = 0.09$). *L. monocytogenes* contamination was also more common among the FPPs that did not perform in-between-process cleaning for the slicing machine than among those performing it (100% vs. 33%, respectively, Fisher's exact test, $p = 0.08$), and a similar difference was observed for the skinning machine (75% vs. 40%, respectively, Fisher's exact test, $p = 0.4$; Table 4). Having a written sanitation plan for the vacuum machine, which was associated with the FPP *L. monocytogenes* status in the univariate (Fisher's exact test, $p = 0.02$, Table 4) but not in the multivariate (chi-squared = 2.2, $p = 0.1$) analyses also appeared to correlate with the in-between-process cleaning of the slicing and skinning machines (Pearson's correlation coefficients 0.5, $p = 0.07$, and 0.6, $p = 0.04$, respectively). However, the in-between-process cleaning of the slicing machine was not associated with *L. monocytogenes* risk in the multivariate analysis (chi-squared = 0.5, $p = 0.5$).

Periodic thorough sanitation refers to sanitation procedures which surpass the extent of everyday cleaning and are performed at particular time intervals. Periodic thorough sanitation was conducted for the processing environment in 20/21 and for the machinery in 14/21 FPPs. Periodic thorough sanitation was performed more often at the *L. monocytogenes* negative than positive FPPs: on average 14 and 3 times per year for the environment (Mann–Whitney *U* test, $p = 0.05$) and 10

Table 4
Presence and absence of processes, procedures, and processing machinery in fish-processing plants (FPPs, n = 21) and proportion of FPPs where *Listeria monocytogenes* (*Lm*) product contamination occurred.

Process, procedure, or processing machine	Present in number of FPPs (proportion of which *Lm* positive, %)	Absent in number of FPPs (proportion of which *Lm* positive, %)	*p* value[c] (Fisher's exact test)
Melting of raw materials	7 (29)	14 (36)	1.0
Head removal	12 (33)	9 (33)	1.0
Head removal machine	1 (100)	20 (30)	0.3
Filleting	15 (60)	6 (17)	0.6
Filleting machine	3 (67)	18 (27)	0.3
Skin present at salting of gravad (n = 18)[a]	11 (27)	7 (57)	0.3
Skin present at salting of cold-smoked	19 (37)	2 (0)	0.5
Skinning	15 (47)	6 (0)	0.06
Skinning machine	14 (50)	7 (0)	0.05
Written sanitation plan for skinning machine	9 (44)	5 (60)	1.0
In-between-process cleaning for skinning machine	10 (40)	4 (75)	0.4
Periodic thorough sanitation for skinning machine	7 (43)	7 (57)	1.0
Slicing	17 (41)	4 (0)	0.3
Slicing machine	15 (47)	6 (0)	0.06
Written sanitation plan for slicing machine	12 (42)	3 (67)	0.6
In-between-process cleaning for slicing machine	12 (33)	3 (100)	0.08
Periodic thorough sanitation for slicing machine	8 (38)	7 (57)	0.6
Vacuum-machine (n = 19)[b]			
Written sanitation plan for vacuum machine	9 (10)	10 (67)	0.02
In-between-process cleaning for vacuum machine	6 (17)	13 (46)	0.3
Periodic thorough sanitation for vacuum machine	12 (17)	7 (72)	0.05
Assigned person in-charge for each self-checking program	15 (20)	6 (67)	0.1
Staff moving from areas of low toward high hygiene during production day			
In all FPPs	14 (43)	7 (14)	0.3
In FPPs with slicing machine (n = 15)	8 (75)	7 (14)	0.04
Cleaning performed during processing in same facilities	6 (50)	15 (27)	0.4
In-between-process cleaning for all machines (n = 19)[b]	6 (17)	13 (46)	0.3
Periodic thorough sanitation for all machines (n = 19)[b]	8 (13)	11 (55)	0.2

[a] 18/21 FPPs produced both gravad and cold-smoked fish products, whereas 3/21 only cold-smoked products.
[b] Data concerning vacuum-packaging machines were obtained from 19/21 FPPs.
[c] Difference in proportion of *Lm* positive FPPs between FPPs where process, procedure, or processing machine are "present" and "absent."

and 5 times per year for the machinery (Mann–Whitney *U* test, $p = 0.4$), respectively (Table 5). The methods used for periodic thorough sanitation included extensive dismantling of machinery, cleaning of structures not washed every day (e.g. roofs), acidic or acid-base wash, long duration of action of sanitizers, and mechanical cleaning. In the multivariate analysis, periodic thorough sanitation of the processing environment was negatively associated with *L. monocytogenes* occurrence in the products: the more often a periodic thorough sanitation was executed, the smaller became the risk of the FPP having had *L. monocytogenes* product contamination (Table 6). In addition, performing a periodic thorough sanitation of the vacuum machine was associated with a decreased risk of product contamination (Table 6). The periodic thorough sanitation of the vacuum machine correlated with the periodic thorough sanitation of the slicing machine (Pearson's correlation coefficient 0.6, $p = 0.01$).

3.5. Views of inspectors on FPP compliance

The opinions of the inspectors concerning the food safety compliance of the FPPs, including the hygiene of FPP operations, functionality of the self-checking system, conformance to official control, and communication with the inspector, were investigated with summed variables consisting of Likert-scale claims (Supp. Table S1). The opinions regarding the conformance to official control were on average poorer in the *L. monocytogenes* positive than negative FPPs, but this difference was not significant in the individual statistical analyses (Table 5). Nonetheless, the inspectors agreed more firmly to having considered the use of coercive measures in the official control of the *L. monocytogenes* positive than negative FPPs (Mann–Whitney *U* test, $p = 0.06$).

4. Discussion

We found the following deficiencies in sanitation practices that were associated with an increased risk of *L. monocytogenes* product contamination: infrequent periodic thorough sanitation of the processing environment and the lack of periodic thorough sanitation of the vacuum machine. The correlation of the latter with the thorough sanitation of the slicing machine (meaning that in FPPs, where a periodic thorough sanitation was performed for one of these machines, it was likely also performed for the other) indicates that the failure to periodically thoroughly clean several of the FPP machines can increase the risk of *L. monocytogenes* product contamination. Sanitation of processing machinery is important in the food industry (Autio et al., 1999; Blatter et al., 2010; Huss et al., 2000; Nakamura et al., 2006; Tolvanen et al., 2009; Tompkin, 2002), but poor hygienic design is perceived to

Table 5
Hygiene parameters and opinions of inspectors on food safety compliance in *Listeria monocytogenes* (*Lm*) positive and negative fish-processing plants (FPPs).

Variable	Average score in *Lm* positive FPPs (variation among them)	Average score in *Lm* negative FPPs (variation among them)	*p* value[d] (Mann–Whitney *U* test)
Hygiene parameter			
Fish skin touching flesh at processing steps[a]	3.4 (2.0–4.7)	3.2 (1.0–6.0)	0.6
Fish skin touching flesh at machinery[a]	2.1 (1.0–4.0)	1.4 (1.0–4.0)	0.3
Hands washed when required[b]	2.5 (1.0–4.3)	3.0 (1.0–5.9)	0.7
Utensils washed when required[b]	2.6 (1.0–5.0)	2.6 (1.0–5.0)	0.8
Gloves changed when required[b]	2.1 (1.0–3.0)	2.5 (1.9–5.0)	0.8
Dirtiness and erosion of processing surfaces[a]	2.1 (1.8–2.9)	1.9 (1.3–2.9)	0.2
Dirtiness and erosion of processing machinery[a]	1.6 (1.0–2.5)	1.5 (1.0–4.0)	0.5
Mechanical cleaning of machinery surfaces[b]	2.8 (1.0–5.0)	2.5 (1.0–6.0)	0.5
Dismantling of machinery during sanitation[b]	3.5 (1.0–5.0)	2.9 (1.0–6.0)	0.5
Proportion of machinery included in *Lm* sampling (%)	21 (0–100)	35 (0–100)	0.4
Periodic thorough sanitation of environment (times/year)	3 (0–12)	14 (1–52)	0.05
Periodic thorough sanitation of machinery (times/year)	5 (0–16)	10 (0–48)	0.4
Summed variable on opinion of compliance			
Hygiene of FPP operations good[c]	1.7 (1.0–2.6)	1.7 (1.0–3.0)	0.9
Functionality of self-checking system good[c]	1.6 (1.0–3.0)	1.8 (1.0–3.8)	0.7
FPP conformance to official food control good[c]	2.0 (1.0–3.8)	1.5 (1.0–2.3)	0.1
Communication between FPP and inspector good[c]	1.7 (1.0–2.3)	1.5 (1.0–2.0)	0.4

FPPs: n = 21, except for "communication" (n = 20) and "machinery" (n = 19).
[a] Extent 1–6: not at all – little – quite little – quite much – much – very much.
[b] Frequency 1–6: always – often – quite often – quite seldom – seldom – never.
[c] Opinion 1–4: completely agree – somewhat agree – somewhat disagree – completely disagree.
[d] Difference between L. *monocytogenes* positive and negative FPPs.

be a problem for several processing machines (Aarnisalo et al., 2006; Giske et al., 2017). We hypothesize that implementation of a periodic thorough sanitation protocol encourages intermittent performance of more meticulous cleaning and dismantling of complex machinery than is achieved during routine everyday sanitation. Therefore, alongside efficient daily sanitation, periodic thorough sanitation likely facilitates the maintenance of an adequate level of cleanliness for *L. monocytogenes* prevention. In a retail setting, a single deep clean prevailed over enhanced daily sanitation procedures at reducing *L. monocytogenes* prevalence in highly contaminated facilities (Etter et al., 2017; Hammons et al., 2017). However, performing periodic thorough sanitation of the processing environment and machinery is currently not mentioned in the national or EU-level fish industry guidelines (European Salmon Smokers Association, 2018; Finnish Food and Drink Industries' Federation, 2006). In light of our results, periodic thorough sanitation should be regarded as an essential measure to be incorporated into the guidelines concerning *L. monocytogenes* prevention.

Cleaning of equipment during daily production has been inferred to decrease the risk of *L. monocytogenes* product contamination (Rørvik et al., 1997). Thereby, effective methods for *L. monocytogenes* prevention by in-between-process cleaning of machinery require further investigation. Our results suggest that in-between-process cleaning, particularly for the slicing and skinning machines and including disinfection, may support the prevention of *L. monocytogenes* contamination, whereas rinsing machines with water during the processing day, as well as cleaning while processing in the same area, might predispose to *L. monocytogenes* contamination. These findings endorse the execution of disinfection and the abandoning of wet clean-ups when performing in-between-process cleaning. However, they also demonstrate that FPPs both rinse with water and perform cleaning while processing, although these are considered poor hygienic practices (Lappi et al., 2004; Tompkin, 2002), and experimental evidence even describes the possibility of *Listeria* cross-contamination by airborne water sprays (Berrang and Frank, 2012). In order to avoid misconceptions, we suggest that industry and authority guidelines and the research community refrain from recommending cleaning "during

Table 6
Results of two (a. and b.) separate generalized linear models (penalized logistic regression) for covariates and factors associated with the occurrence of *Listeria monocytogenes* in the products of fish-processing plants (FPPs).

Model attribute or parameter	Deviance	Df	chi-squared	*p* value	β	SE for β	OR	CI 95%
a.								
Null deviance	16.2	20						
Residual deviance	5.6	18	10.6	0.005				
Intercept				0.004	−5.18	2.83		
Periodic thorough sanitation for the processing environment (times/year)		1	6.4	0.01	−0.11	0.059	0.90	0.71–0.98
Number of processing machines		1	8.9	0.003	1.91	1.01	6.7	1.6–1730
b.								
Null deviance	19.6	14						
Residual deviance	10.5	12	9.1	0.01				
Intercept				0.01	−2.72	1.53		
Periodic thorough sanitation for vacuum machine (yes vs. no)		1	4.3	0.04	2.70	1.63	15	1.03–2060
Staff movement from areas of low toward high hygiene during production day (no vs. yes)		1	4.5	0.03	2.71	1.61	15	1.1–2100

SE = standard error; OR = odds ratio; CI = confidence interval.
a. All FPPs (n = 21, pseudo-R^2 = 65%).
b. FPPs with slicing machine (n = 15, pseudo-R^2 = 46%).

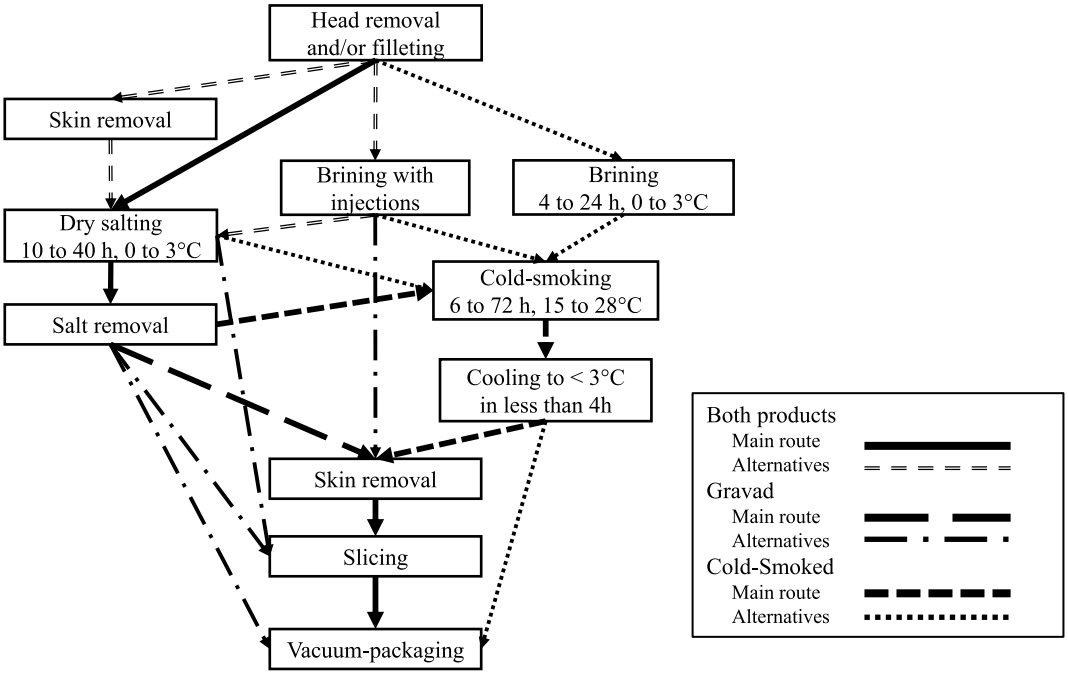

Fig. 1. Processing steps in the production of gravad and cold-smoked vacuum-packaged fish in the studied fish-processing plants.

processing" but "during processing pauses" instead.

An increase in the number of processing machines increased the risk of product contamination with *L. monocytogenes*, which likely explains why the bacterium mainly occurred in the products of the large and medium-sized FPPs. The number of processing machines varied primarily due to the presence or absence of skinning and slicing machines, and *L. monocytogenes* only occurred in the FPPs where these machines were utilized. Both machines can harbor *L. monocytogenes* contamination in FPPs (Autio et al., 1999; Chitlapilly Dass et al., 2010; Di Ciccio et al., 2012; Gudmundsdottir et al., 2005; Nakamura et al., 2006; Thimothe et al., 2004; Vogel et al., 2001); therefore, particular attention should be paid to *L. monocytogenes* in the FPPs utilizing these machines. However, our results illustrate that *L. monocytogenes* prevention was currently not at a sufficient level in many such FPPs.

Staff movement from processing areas of low hygiene toward areas of high hygiene during a working day was found to be associated with an increased risk of product contamination with *L. monocytogenes* in the FPPs operating a slicing machine, i.e., in relatively large FPPs. Spatial or temporal arrangements preventing the movement from areas of low toward high hygiene should be in place, such as hygiene barriers or temporal separation of processes in a descending hygienic order. Appropriate infrastructure and resources available for hygienic practices support the continuous prevention of *L. monocytogenes* (Clayton et al., 2002; Hicks et al., 2004). The current results thus emphasize how *L. monocytogenes* must be considered when building or renovating facilities and designing hygienic routes therein. These considerations include compartmentalizing processing lines and avoiding job rotation during the processing day, which are associated with *L. monocytogenes* contamination (Lundén et al., 2003; Rørvik et al., 1997).

During the 14-month follow-up, the products of seven FPPs tested positive for *L. monocytogenes*, while each of these FPPs exhibited their own *L. monocytogenes* pulsotypes. Product contamination occurred more than once in three FPPs, in each of which their own same pulsotype was found on the separate sampling occasions. Although L. *monocytogenes* occasionally occurs in raw materials and FPPs, recurrent appearance of the same pulsotypes can indicate persistent contamination (Autio et al., 2004; Chitlapilly Dass et al., 2010; Di Ciccio et al., 2012; Eklund et al., 1995; Markkula et al., 2005; Vogel et al., 2001). *L. monocytogenes* only occurred in sliced products and was more prevalent in gravad than cold-smoked fish. The latter could be due to drying of the fish surface or the presence of antibacterial compounds during the smoking process, which can deter *L. monocytogenes* growth (Hwang et al., 2009; Porsby et al., 2008). However, the role of non-sliced or cold-smoked fish as vehicles must not be underestimated, as such products have also been reported to contain *L. monocytogenes* and have been implicated in outbreaks (Ericsson et al., 1997; Gillesberg Lassen et al., 2016; Miettinen et al., 1999; Nakari et al., 2014).

All product samples were in compliance with the EU legislative limit of 100 cfu/g during their shelf life (EC No 2073/2005). The low observed counts of *L. monocytogenes* can be explained not only by a potentially low level of initial contamination but also by storage at 3 °C, where growth takes several days (Markkula et al., 2012; Pöntinen et al., 2015). Retail and consumer refrigerators, however, are often kept at above 3 °C (James et al., 2008; Lundén et al., 2014). Storage of vacuum-packaged gravad and cold-smoked products is recommended at < 3 °C for a maximum of 14 days by the Finnish Food Authority (the Finnish Food Safety Authority Evira by former name). In our study, no association between product shelf life and *L. monocytogenes* contamination was observed, although not all consume-by dates fell within the recommendation. Nonetheless, the observed variation of shelf life by several days in the same FPP for the same type of product raises uncertainty about whether the shelf lives were based on arbitrary or experimentally validated principles. Since determining the duration of the shelf life is up to the manufacturer, it can be based on other principles than rigorous experimental evidence or challenge tests.

The occurrence of *L. monocytogenes* in fish products was associated with their reportedly high salt content. This might be explained by a competitive advantage gained by *L. monocytogenes* in saline conditions, if the growth of other bacterial populations acting as natural protective cultures was inhibited (Gimenez and Dalgaard, 2003; Jorgensen and Huss, 1998). However, the confirmation of this hypothesis requires further investigation. Brine and injection brining have previously been identified as risk factors for *L. monocytogenes* (Autio et al., 1999; Bērziņš et al., 2007; Gudmundsdottir et al., 2005; Vogel et al., 2001), but in our study mostly dry salting was used by FPPs. Product contamination appeared to be more common among the FPPs where skinning had been performed before, as opposed to after, salting of gravad products, although this putative link requires further research. High salt amounts,

dry salting, and early skinning are listed as listerial preventive measures in a recently published guidance document on good practice for European smoked, salted, and marinated fish products (European Salmon Smokers Association, 2018). Nevertheless, these measures did not appear to prevent *L. monocytogenes* contamination in our investigation and require further consideration in order to ensure that the guidelines are up-to-date.

The prevention of *L. monocytogenes* product contamination must consist of stringent, continuous efforts (Autio et al., 1999; Hu et al., 2006; Lappi et al., 2004), which calls for the full implementation of effective cleaning, hygiene, and monitoring practices. The monitoring of cleaning results should contain *L. monocytogenes* self-checking sampling from all processing machines, which was on average relatively uncommon in the studied FPPs. Conversely, with only a few exceptions, cleanliness of hands, utensils, and gloves was achieved by staff as required by inspectors in all FPPs. *L. monocytogenes* contamination of FPP personnel aprons, hands, and gloves has been reported during processing (Thimothe et al., 2004), but in the current study, no statistical associations were observed between the reported working hygiene parameters and *L. monocytogenes* product contamination. However, our findings imply that assigning a person in charge of each self-checking system program might be associated with *L. monocytogenes* prevention. Such allocated responsibility could lead to enhanced compliance through improved commitment and is consistent with the attentive management culture required in the execution of food safety practices described by Clayton et al. (2002).

Knowledge of food safety and attitudes toward official control can be reflected in the hygiene practices of food industry operators (Davies et al., 2014; Läikkö-Roto and Nevas, 2014; Yapp and Fairman, 2006). In this investigation, the official food inspectors were of the opinion that the *L. monocytogenes* positive FPPs conformed somewhat less to official control than the *L. monocytogenes* negative ones, implying that a negative attitude may have influenced their *L. monocytogenes* prevention. The more pronounced consideration of enforcement measures by the inspectors for the *L. monocytogenes* positive than negative FPPs indicates likewise. We have discovered that FPPs with recurrent *L. monocytogenes* problems exhibited difficulties collaborating with official food control authorities (Aalto-Araneda et al., 2018). The results of the current study further emphasize the importance of incorporating motivation-building and supportive cooperation with official control into the *L. monocytogenes* preventive measures of FPPs.

5. Conclusions

This study of the implementation of *L. monocytogenes* preventive measures in a representative sample of FPPs was the first in-depth analysis, which found several significant associations between operational features and *L. monocytogenes* occurrence in products. Processing machinery (particularly the slicing and skinning machines), and deficiencies in sanitation and hygiene practices were identified as risk factors for *L. monocytogenes* contamination in vacuum-packaged RTE fish products. *L. monocytogenes* contamination occurred only in sliced products and was significantly more common in gravad than in cold-smoked fish. Our results indicate that hygiene measures important for *L. monocytogenes* prevention have not been carried out efficiently in all FPPs. Specifically, improvements in hygienic routes and thorough sanitation of the processing environment and machinery can enhance the prevention of *L. monocytogenes* product contamination. These improvements emphasize the commitment and continuity required for *L. monocytogenes* prevention.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.03.017.

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